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# Treatment with Cestode Parasite Antigens Results in Recruitment of CCR2$^+$ Myeloid Cells, the Adoptive Transfer of Which Ameliorates Colitis

**José L. Reyes,[a,b] Fernando Lopes,[a] Gabriella Leung,[a] Nicole L. Mancini,[a] Chelsea E. Matisz,[a] Arthur Wang,[a] Emma A. Thomson,[a] Nicholas Graves,[a] John Gilleard,[c] Derek M. McKay[a]**

Gastrointestinal Research Group, Department of Physiology and Pharmacology, Calvin, Joan and Phoebe Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada[a]; Laboratorio de Immunología Experimental y Regulación de la Inflamación Hepato-Intestinal, UBIMED, FES Iztacala, UNAM, Estado de Mexico, México[b]; Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada[c]

**Awareness of the immunological underpinnings of host-parasite interactions may reveal immune signaling pathways that could be used to treat inflammatory disease in humans. Previously we showed that infection with the rat tapeworm, *Hymenolepis diminuta*, used as a model helminth, or systemic delivery of worm antigen (HdAg) significantly reduced the severity of dinitrobenzene sulfonic acid (DNBS)-induced colitis in mice. Extending these analyses, intraperitoneal injection of HdAg dose-dependently suppressed dextran sodium sulfate (DSS)-induced colitis, and this was paralleled by reduced gamma interferon (IFN-γ), interleukin-17 (IL-17), and tumor necrosis factor alpha (TNF-α) production and increased IL-10 production from mitogen-activated splenocytes. Treatment with HdAg resulted in a CCR2-dependent recruitment of CDllb$^+$ F4/80$^+$ Ly6C$^{hi}$ Gr-1$^{lo}$ monocyte-like cells into the peritoneum 24 h later that were predominantly programmed death ligand 1 (PD-L1) positive and CXCR2 negative. *In vitro* assays indicated that these cells were unable to suppress T cell proliferation but enhanced IL-10 and IL-4 production from activated T cells. Adoptive transfer of the HdAg-recruited monocytic cells into naive mice blocked DSS-induced colitis. These findings add to the variety of means by which treatment with parasitic helminth-derived antigens can ameliorate concomitant disease. A precise understanding of the mechanism(s) of action of HdAg and other helminth-derived antigens (and a parallel consideration of putative side effects) may lead to the development of novel therapies for human idiopathic disorders such as inflammatory bowel disease.**

Until relatively recently, analysis of the host response to infection with helminth parasites focused almost invariably on TH2 immunity; however, it has emerged that helminth parasites trigger a complex regulatory network in their mammalian hosts that is characterized by cytokines (e.g., interleukin-10 [IL-10] and transforming growth factor β [TGF-β]) and cellular components (e.g., regulatory macrophages and T cells) (1). Indeed, the development of an immunoregulatory environment likely contributes to the chronicity of helminth infection and asymptomatic disease. Moreover, individuals infected with a variety of species of helminths can be protected from concomitant disease as demonstrated in animal models of multiple sclerosis (2–4), joint (5–7) and gut (8–10) inflammation, and allergy (11, 12). In addition, treatment with somatic extracts or secreted products can significantly attenuate disease severity in models of inflammatory diseases (13–15), raising the possibility that isolation and purification of helminth-derived molecules could result in new anti-inflammatory drugs.

The inverse relationship between the geographical distribution of inflammatory bowel disease (IBD) (i.e., Crohn's disease and ulcerative colitis) and areas of endemic helminth infection suggests that infection with helminth parasites may protect against IBD (16). Testing this hypothesis, infections with *Trichinella spiralis*, *Schistosoma mansoni*, and *Heligmosomoides polygyrus* were shown to inhibit inflammation in dinitrobenzene sulfonic acid (DNBS)- and dextran-sodium sulfate (DSS)-induced colitis and piroxicam-treated IL-10$^{-/-}$ mice, respectively (8, 9, 17)—all established mouse models of colitis that share some similarities to human IBD. Similarly, and as an alternative to viable infection, systemic administration of helminth-derived antigens can ameliorate colitis in animal models. As examples, the excretory/secretory (E/S) products from adult *T. spiralis* reduced DSS-induced colitis (18) and *S. mansoni* egg antigens ameliorated immune-mediated colitis (19): in both instances, suppression of TH1 and TH17 cytokines correlated with the beneficial anticolitic effect. While encouraging, the precise mechanism of action of any helminth-derived extract or molecule to block colitis or other inflammatory diseases is not well understood.

In some of the first studies on helminth-induced suppression of colitis, we found that mice infected with five cysticercoids of the rat tapeworm, *Hymenolepis diminuta*, showed minor improve-

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Address correspondence to José L. Reyes, joseluis611@yahoo.com.mx, or Derek M. McKay, dmckay@ucalgary.ca.

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ment in DSS-induced disease (20) but were remarkably protected from the colitic effect of intrarectal administration of DNBS (10). Daily intraperitoneal (i.p.) doses (1 mg) of a high-molecular-weight (MW) crude extract of adult *H. diminuta* over the 3 days of DNBS treatment significantly reduced the severity of inflammation in the colon (21). The relatively minor ability of infection with *H. diminuta* to alleviate DSS-induced disease was puzzling. Consequently, we tested the hypothesis that a crude extract of adult *H. diminuta* antigens (HdAg) would attenuate colitis induced by DSS.

The data herein reveal that HdAg treatments significantly reduce the severity of DSS colitis; intraperitoneal delivery of the HdAg resulted in recruitment of $CCR2^+$ $PD\text{-}L1^+$ monocyte-like cells. Analysis of these $CCR2^+$ $PD\text{-}L1^+$ $F4/80^+$ $Ly6C^+$ $Gr\text{-}1^{lo}$ cells revealed their capacity to induce IL-10 secretion by T cells. Adoptive transfer of these cells inhibited DSS-induced colitis in the recipient mice, indicating the potential for helminth-evoked $CCR2^+$ $PD\text{-}L1^+$ $F4/80^+$ $Ly6C^+$ $Gr\text{-}1^{lo}$ cells to suppress intestinal inflammation.

## MATERIALS AND METHODS

**Ethics.** All of the experiments conducted in this study conformed to Canadian national guidelines on animal use in experimentation as administered by the Health Science Animal Care Committee under ethics protocol AC-13-005.

**Generation of *H. diminuta* crude antigens (HdAg).** Adult *H. diminuta* parasites were flushed from the small intestine of rats (Charles River, QC, Canada) with sterile phosphate-buffered saline (PBS), treated with antibiotics (gentamicin solution; Sigma, St. Louis, MO]) for 2 h, centrifuged, and then homogenized in sterile PBS on ice using a Polytron PT1200 (Kinematica AG, Switzerland). The homogenate was centrifuged twice at 4,000 rpm for 30 min at 4°C, the PBS-soluble supernatant was collected, and the pellet was discarded. Endotoxin measurement (ToxinSensor Chromogenic LAL kit; GenScript, Piscataway, NJ) revealed 65 pg lipopolysaccharide (LPS)/1 mg of HdAg extract. The protein concentration in the HdAg preparations was determined by the Bradford assay (Bradford reagent; Sigma-Aldrich, St. Louis MO), and aliquots were stored at −80°C. Three separate HdAg preparations were used in this investigation, and each suppressed LPS-induced tumor necrosis factor alpha (TNF-α) production from the THP-1 monocytic cell line by at least 40% (21).

**Induction and assessment of murine colitis.** Male 7- to 9-week-old BALB/c or C57BL/6 mice (Charles River) were divided into four experimental groups: (i) mice receiving tap water (water group), (ii) mice receiving 5% (wt/vol) (BALB/c) or 3% (wt/vol) (C57BL/6) DSS (MW, 40,000 to 50,000 [Affymetrix]) in drinking water for 5 days with an additional 3 days on normal tap water (DSS group) (or given i.p. injections of sterile PBS as a control), (iii) mice exposed to DSS and given different doses of HdAg via i.p. injection in 500 μl of sterile PBS at indicated time points (Fig. 1A) (DSS+HdAg group), and (iv) mice injected with HdAg only (HdAg group). In other experiments, CCR2-deficient mice (C57BL/6 genetic background; provided by P. Kubes, University of Calgary) were used and compared to wild-type C57BL/6 mice. Mouse body weight was recorded daily, and at necropsy, the colon was removed and measured, and a macroscopic disease activity score (DAS) was calculated based on a validated scoring system (10). Additionally, a portion of mid-colon was formalin fixed and processed to wax, and a histological damage score was calculated on 5-μm hematoxylin and eosin (H&E)-stained sections in a blind fashion as described previously (10, 22).

In adoptive transfer studies, $1 \times 10^6$ sorted cells were given in 500 μl sterile PBS via i.p. injection on days 2 and 4 post-DSS. Necropsy to assess colitis was conducted on day 8. An additional study was conducted in which cells ($10^6$) isolated from the peritoneal cavity 24 h after HdAg were treated with a neutralizing PD-L1 antibody (20 μg/ml; Biolegend, San Diego, CA) for 2 h at 37°C prior to adoptive transfer.

**Splenocyte cultures.** Spleens were aseptically removed, photographed, weighed, and then mechanically dispersed into a single-cell suspension, and red blood cells were lysed with ammonium chloride buffer. Leukocytes were resuspended in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS), 1.2% GlutaMAX, and 2.4% penicillin-streptomycin (Gibco, CA) at $1 \times 10^6$ cells/ml for flow cytometry staining and $5 \times 10^6$ cells/ml for stimulation with concanavalin A (ConA [Sigma Chemical Co.]).

**Cytokine measurements by ELISA.** Forty-eight hours after ConA (5 μg/ml) stimulation, immune cell supernatants were collected, and levels of IL-4, IL-10, IL-17A, gamma interferon (IFN-γ), and TNF-α were determined by enzyme-linked immunosorbent assay (ELISA) using paired antibodies and following the manufacturer's instructions (R&D Systems, Minneapolis, MN).

**Isolation of PECs.** Four or 24 h after i.p. injection of HdAg, peritoneal exudate cells (PECs) were retrieved by lavage with 10 ml of sterile PBS. Cell viability was assessed by trypan blue exclusion, and viable cells were adjusted to $10^6$/ml to perform flow cytometry. PECs from LPS-injected mice (1 μg) were used as a comparison with HdAg (65 pg contaminating LPS/1 mg HdAg).

**Flow cytometry analysis and cell sorting.** Splenic T cells or PECs were first incubated with anti-mouse CD16/32 TruStain FcX antibodies (Biolegend, San Diego, CA) at 4°C for 15 min to prevent unspecific antibody binding and then stained with allophycocyanin (APC)-F4/80, phycoerythrin (PE)–Gr-1, peridinin chlorophyll protein (PerCP)-CXCR2, APC–PD-L1, PE–PD-L2, fluorescein isothiocyanate (FITC)–IL-10 (Biolegend, San Diego, CA), Alexa Fluor 450-CD11b, APC/Cy7-Ly6C (BD Bioscience, San Jose, CA), PE/Cy7-F4/80 (eBioscience, San Diego, CA), and PE-CCR2 (R&D Systems, Minneapolis, MN) at 4°C for 30 min, washed twice in flow cytometry buffer (PBS with 1% FBS and 0.1% sodium azide; Sigma Chemical Co.), and analyzed using an Attune flow cytometer with flow cytometric software version 1.2.5 (R&D systems). Cells were first gated based on forward and side scatter (FSC and SSC, respectively) attributes, and doublets were excluded by FSC width and FSC height characteristics (22).

In additional experiments, cell sorting of $F4/80^+$ $Gr\text{-}1^{lo}$ cells was conducted at the University of Calgary Flow Cytometry Core Facility using the FACSAria II machine and FACSDiva version 6.1.3 software (BD Biosciences). Sorted cells were washed in RPMI 1640 medium with 1% FBS, 1.2% GlutaMAX, and 2.4% penicillin-streptomycin for use in either *in vitro* cocultures or adoptive transfer studies. Sorted cells were assessed for Ly6C expression. Also, sorted cell ($5 \times 10^4$) cytospins were stained with modified Giemsa-Wright solution and observed via light microscopy.

**T cell-HdAg-recruited monocytic cells *in vitro* cocultures.** Splenocytes isolated from naive mice were stained with carboxyfluorescein succinimidyl ester (CFSE; 5 μM [CellTrace cell proliferation kit; Life Technologies, Grand Island, NY]) for 10 min at 37°C. The reaction was quenched with 20 ml of RPMI medium supplemented with FBS (20%). Thereafter, magnetic isolation of pure $CD4^+$ splenic T cells was performed according to the manufacturer's instructions (EasySep mouse negative selection, $CD4^+$ T cell enrichment kit; Stem Cell Technologies, Inc., Vancouver, BC, Canada). Twenty-four-well plates were coated overnight with anti-CD3 antibodies (2 μg/ml [Biolegend, San Diego, CA]) in sterile PBS, and 24 h later, purified CFSE-stained $CD4^+$ T cells ($1 \times 10^6$/ml) were added along with soluble anti-CD28 antibodies (1 μg/ml [Biolegend]) and incubated for 2 h (37°C, 5% $CO_2$). Next, PECs or sorted $F4/80^+$ $Gr\text{-}1^{lo}$ monocytic cells recruited in response to HdAg (with peritoneal cells from PBS-treated mice used as controls) were added at a 1:4 ratio of PECs to T cells and coincubated for 96 h. To determine proliferation, CD4-specific staining was conducted, and cells gated on CD4 were assessed for CFSE dilution. In addition, cell culture supernatants were collected, and nitrites and cytokines were measured. In other experiments, either the inducible nitric oxide synthase (iNOS) inhibitor nitro-

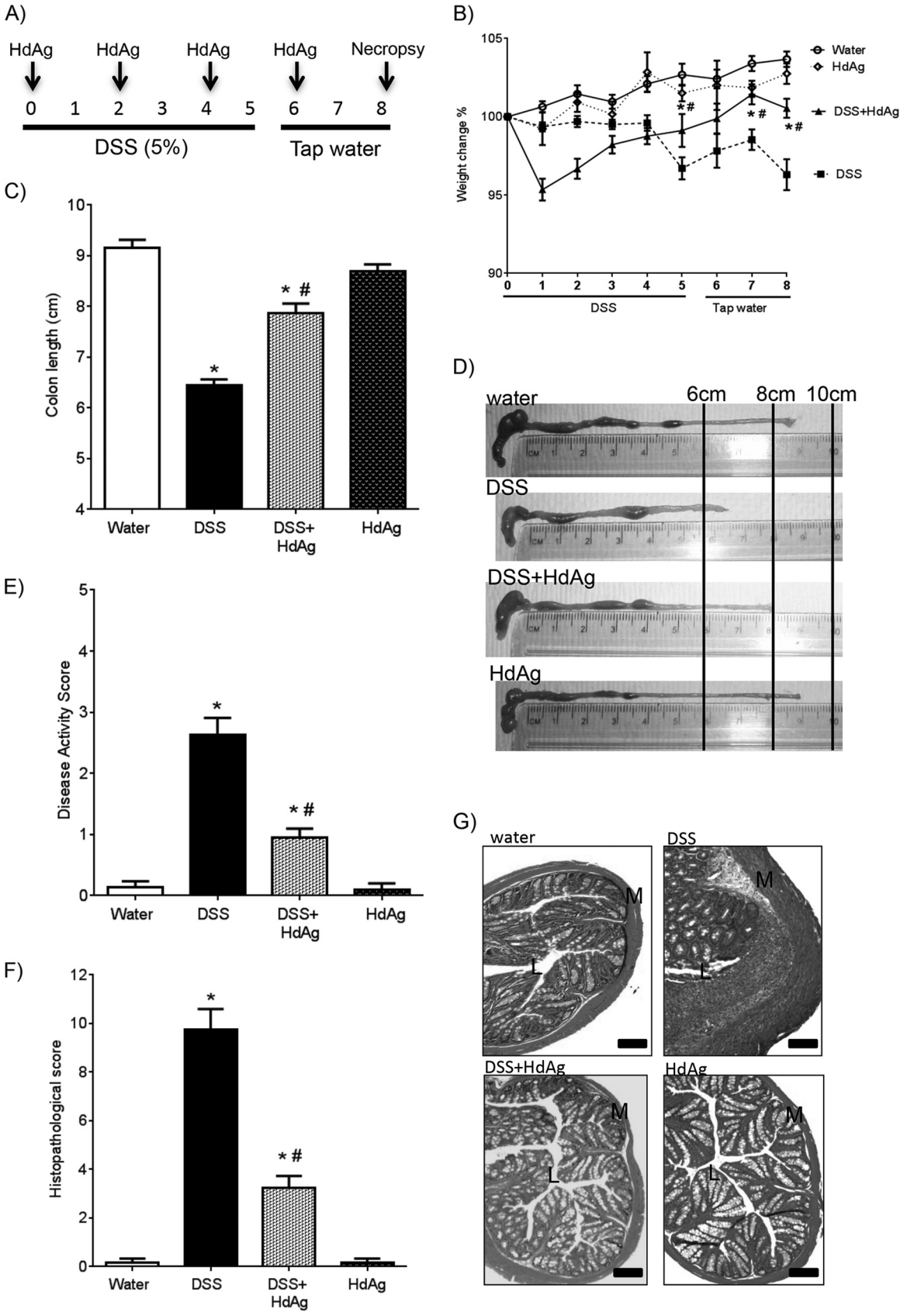


**FIG 1** Intraperitoneal delivery of *H. diminuta* antigens inhibits DSS-induced colitis. Panel A shows the 5% (wt/vol) DSS and HdAg (1 mg i.p.) treatment regimen. Panel B shows the change in percentage of weight over the 8 days. On necropsy, colon length was recorded (C and D), a composite macroscopic disease activity score was calculated (E), and colonic histopathology was assessed (F and G). Data are means ± SEM from three independent experiments with $n = 9$ to 13 mice. Representative H&E-stained sections of midcolon are shown (scale bars, 100 μm). L, lumen; M, mucosa. * and #, $P < 0.05$ compared to mice treated with water only and mice treated with DSS, respectively.

**TABLE 1** HdAg reduces the severity of DSS-induced colitis after 5 days of treatment

| Exptl group[a] | Result for parameter[b]: | | |
|---|---|---|---|
| | Colon length (cm) | DAS | Histopathology score |
| Water | 8.6 ± 0.1 | 0 | 0 |
| HdAg | 8.4 ± 0.3 | 0.2 ± 0.2 | 0 |
| DSS | 7.2 ± 0.2* | 1.6 ± 0.4* | 3.0 ± 0.8* |
| DSS+HdAg | 8.2 ± 0.3# | 0.5 ± 0.2# | 1.0 ± 0.3*# |

[a] DSS, dextran sodium sulfate at 5% (wt/vol) for 5 days; HdAg, PBS-soluble *H. diminuta* antigens at 1 mg i.p. on alternate days.
[b] DAS, disease activity score. Data are means ± SEM from 2 experiments ($n = 7$ mice). * and #, $P < 0.05$ compared to the groups treated with water only and DSS, respectively.

L-arginine methyl ester (L-NAME; 1 mM [Sigma-Aldrich]) or a neutralizing anti-PD-L1 antibody (5 μg/ml [Biolegend]) was added to the PEC-T cell cocultures. In PBS-treated mice, there was an absence of recruited F4/80$^+$ Gr-1$^{lo}$ cells, and so F4/80$^+$ Gr-1$^-$ cells were retrieved from the peritoneal cavity and termed "resident" cells for comparison with the HdAg-recruited cells.

**Griess reaction.** Nitric oxide production was determined by measuring nitrite levels in cell culture supernatants as previously reported (23). Supernatants were combined with an equal volume of 2% sulfanilamide and 0.1% *N*-1-naphthylethylenediamine dihydrochloride (both Sigma Chemical Co.) to convert nitrite into a magenta-colored azo compound with a measurable absorbance at 540 nm. Nitrite levels were determined based on a standard curve of known sodium nitrite concentrations.

***In vitro* chemotaxis assay.** Murine bone marrow cells were isolated and stimulated with macrophage colony-stimulating factor (M-CSF [R&D Systems]) for 48 h, and the resultant macrophages were collected and used ($1 \times 10^5$) in an agarose gel chemotaxis assay as previously described (24), using HdAg (1 mg/ml) or LPS (10 μg/ml) as a positive control. Chemotaxis was assessed after 18 h of culture.

**Statistical analysis.** Data are presented as means ± standard errors of the means (SEM). Differences between groups were determined by one-way analysis of variance (ANOVA). When $P$ was <0.05, the ANOVA was followed by *post hoc* analyses with Tukey's multiple-comparison test for parametric data and Dunn's multiple-comparison test for nonparametric data, where $P$ is <0.05 was accepted as a statistically significant difference. All statistical analyses were performed using Graph Pad Prism V5 software.

## RESULTS

**Intraperitoneal delivery of HdAg attenuates DSS-induced colitis, concomitant with reduced TH1 and TH17 cytokine production.** BALB/c mice exposed to drinking water containing 5% DSS (Fig. 1A) developed colitis (Fig. 1B to G). In comparison, mice treated with HdAg (1 mg) on alternate days had significantly less DSS-induced disease as assessed by body weight (Fig. 1B), colon length (Fig. 1C and D), a macroscopic disease score (Fig. 1E), and histological damage/inflammation in the colon (Fig. 1F and G). (C57BL/6 mice treated with HdAg displayed less DSS-induced colitis [see Fig. S1 in the supplemental material].) Unexpectedly, DSS+HdAg-treated BALB/c (Fig. 1B) (but not C57BL/6 mice) showed a drop in weight 24 to 28 h after treatment that rebounded: the reason for this is unexplained. Additional experiments were conducted in which mice received DSS-water for 5 days ± 3 i.p. injections of HdAg every other day, with necropsy on day 5: this resulted in significantly less disease (Table 1), suggesting that early inflammatory events elicited by DSS are blocked by HdAg treatment. Time-matched mice treated with only HdAg showed no signs of disease or histopathology in the colon (Fig. 1; Table 1).

Animals treated with four doses of HdAg ± DSS displayed splenomegaly (Fig. 2A). Cytokine output from mitogen-activated splenocytes retrieved at the end of the DSS exposure (i.e., day 5) or after an additional 3 days of normal drinking water (i.e., day 8) was assessed. Peak production of IFN-γ, IL-17, and TNF-α occurred at day 5 in the DSS group: the levels of these cytokines were significantly lower in the DSS+HdAg group at this time point (Fig. 2B to D). The increases in these cytokines at day 8 were also significantly smaller in DSS+HdAg-treated mice. Stimulated splenocytes from DSS+HdAg-treated and DSS-treated mice produced more IL-10 than cells from control mice at day 5. However, at the 8-day time point, splenocytes from HdAg±DSS-treated mice produced significantly more IL-10 than cells from mice in the other groups (Fig. 2E); IL-4 was similarly increased in these two groups of mice at the 8-day time point (Fig. 2F). HdAg treatment alone, in the absence of disease, significantly increased splenocyte production of IL-17 and TNF-α on days 5 and 8, respectively (Fig. 2C and D), which may be related to the low level of LPS contamination or other Toll-like receptor (TLR) ligands in the crude worm extract.

Flow cytometry revealed a trend toward decreased Foxp3 expression in splenic CD4$^+$ cells from DSS-treated mice that was not statistically significant. DSS+HdAg treatment did not result in any increase in CD4$^+$ Foxp3$^+$ splenocytes ($n = 4$ [data not shown]).

Dose-response analysis (the regimen depicted in Fig. 1A) revealed that 100 μg HdAg had, at best, a mild benefit in inhibiting DSS-induced colitis, whereas repeated 500-μg and 1-mg doses significantly reduced the severity of DSS-induced disease by all indices measured, elicited increases in spleen size and reduced spleen cell production of IFN-γ, IL-17, and TNF-α, and enhanced IL-4 and IL-10 output (Table 2; see Fig. S2 in the supplemental material).

IL-10 is an important immunoregulatory cytokine in enteric inflammation (25, 26); intracellular staining revealed small, statistically nonsignificant percentage increases in IL-10$^+$ splenic lymphocytes (data not shown); however, because of the splenomegaly, this translated into significantly increased numbers of CD4$^+$, CD8$^+$, and CD19$^+$ cells capable of synthesizing IL-10 (Fig. 3).

**Monocyte-like cells recruited in response to HdAg can induce IL-10 production in CD4$^+$ T cells.** Treatment with HdAg could ameliorate colitis via a variety of mechanisms (1, 21, 22). Antigens from the flatworms *S. mansoni* and *Taenia crassiceps* can mobilize myeloid-derived suppressor cells (MDSCs) (27, 28). Assessing peritoneal cellularity, an obvious immune cell recruitment was noted 24 h after HdAg or LPS injection (Fig. 4A): total cell numbers increased 3- to 4-fold from (2.5 ± 0.2) × $10^6$ in PBS-treated mice to (8.4 ± 0.8) × $10^6$ in animals given HdAg (24 h; $n = 10$, $P < 0.01$), with a striking increase in CD11b$^+$ Gr-1$^{lo}$ cells in the HdAg group (Fig. 4B). CD11b and Gr1 identify a number of myeloid and neutrophil populations. Thus, subsequent flow cytometry used Ly6C, F4/80, and CCR2 to distinguish between neutrophils and monocytes/macrophages (Fig. 4C and D). Ly6C and CCR2 staining revealed recruitment of a monocyte-like population with the phenotype CD11b$^+$ Gr-1$^{lo}$ Ly6C$^{hi}$ F4/80$^+$ CCR2$^+$ in the peritoneal cavity 24 h post-HdAg treatment, which is unequivocally identified as an F4/80$^+$ Gr-1$^{lo}$ population (~99%); this was

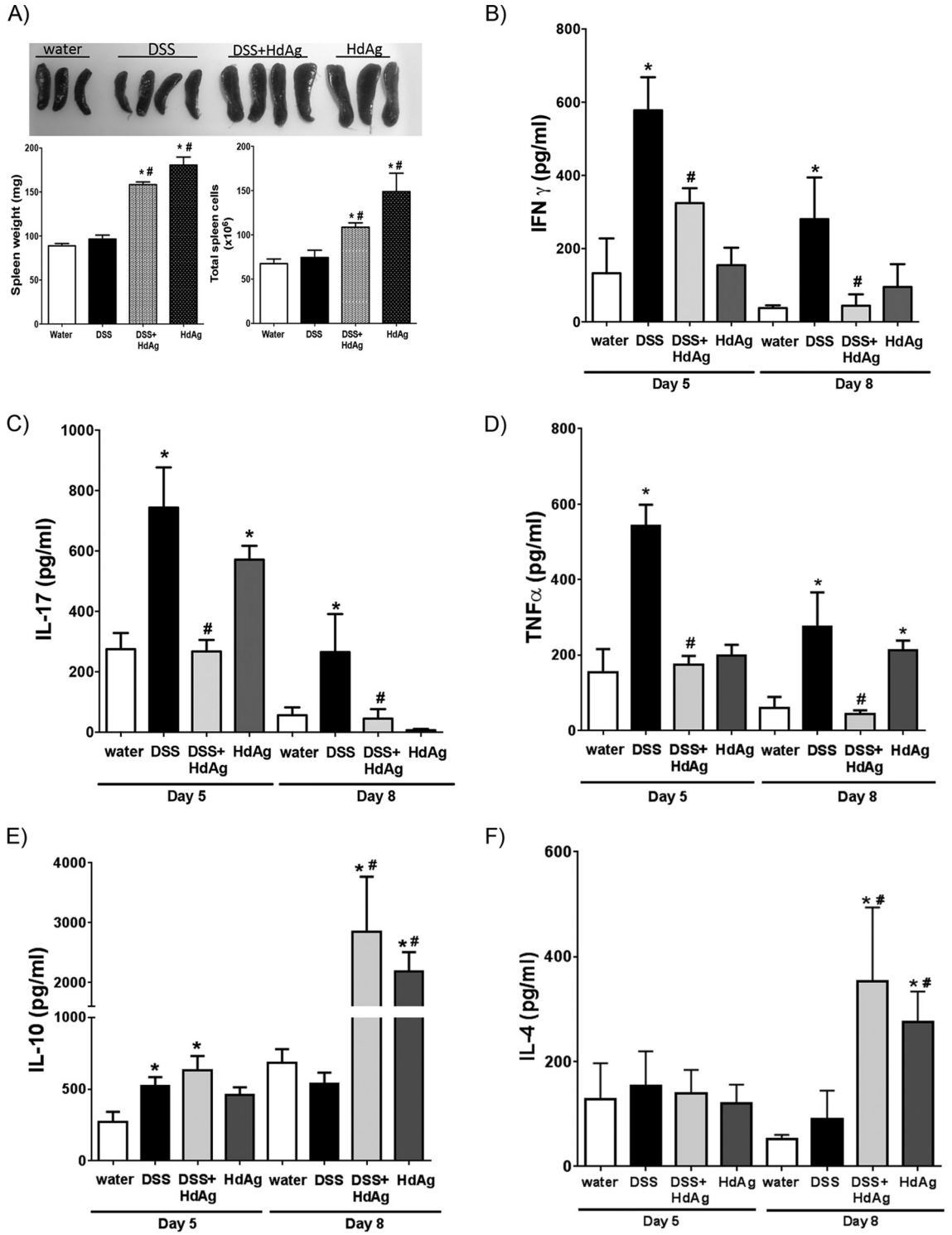


**FIG 2** Treatment with *H. diminuta* antigens reduces inflammatory cytokines in the spleen and promotes IL-10 production. BALB/c mice were treated as in Fig. 1A (5% DSS, 1 mg HdAg), and at necropsy on days 5 and 8, spleens were excised and weighed (A [day 8]), splenocytes were isolated and stimulated with ConA (5 μg/ml per $5 \times 10^6$ cells), and 48 h later, cytokines were measured in the culture medium by ELISA (B to F). Data are means ± SEM from 3 independent experiments with $n = 9$ mice. * and #, $P < 0.05$ compared to mice treated with water only and mice treated with DSS, respectively.

not observed following LPS injection (Fig. 4D). This F4/80$^+$ Gr-1$^{lo}$ population was not observed in the peritoneal cavity 4 h after HdAg treatment, when neutrophil influx was apparent (see Fig. S3 in the supplemental material). Stained cytospin preparations of sorted F4/80$^+$ Gr-1$^{lo}$ cells revealed morphology consistent with a macrophage-type phenotype, whereas the sorted F4/80$^-$Gr-1$^{hi}$ cells had a multilobed nucleus consistent with neutrophils (Fig. 4E) (since the F4/80 marker excludes any neutrophil

**TABLE 2** HdAg regulation of mitogen-stimulated cytokine production by splenocytes

| Cytokine | Cytokine production (pg/ml) in exptl group[a]: | | | |
|---|---|---|---|---|
| | | DSS+HdAg | | |
| | DSS (5% [wt/vol]) | 100 μg | 500 μg | 1 mg |
| IFN-γ | 2,376 ± 492 | 1,113 ± 179* | 1,091 ± 187* | 691 ± 80* |
| IL-17 | 17,867 ± 275 | 911 ± 191* | 401 ± 80* | 322 ± 35* |
| TNF-α | 178 ± 18 | 113 ± 25 | 47 ± 9* | 58 ± 14* |
| IL-4 | 48 ± 3 | 52 ± 7 | 89 ± 15* | 112 ± 17* |

[a] DSS, dextran sodium sulfate at 5% (wt/vol) for 5 days; HdAg, PBS-soluble *H. diminuta* antigens given at 100 μg, 500 μg, or 1 mg i.p. on alternate days. Data are means ± SEM from 2 experiments ($n = 7$ mice). *, $P < 0.05$ compared to the DSS-treated group.

contamination); these cells were predominantly PDL-1$^+$ PDL-1$^-$ (Fig. 4E). We focused on these F4/80$^+$ Gr-1$^{lo}$ recruited monocytic cells [denoted M$^{(HdAg)}$].

M$^{(HdAg)}$ cells retrieved 24 h after treatment did not affect splenic CD4$^+$ T cell proliferation (Fig. 5A); however, there were significant increases in IL-10 and IL-4 in the cocultures (Fig. 5B) that were not observed when the F4/80$^+$ Gr-1$^-$ cells from control PBS-treated mice were used in the cocultures. Given that the M$^{(HdAg)}$ cells spontaneously produced neither IL-10 nor IL-4 in culture (Fig. 5B), and in the context of the splenic cytokine production from *in vivo* analyses, it is likely that the CD4$^+$ T cells were induced to produce both IL-10 and IL-4 in these cocultures.

**HdAg recruitment of monocytic cells to the peritoneal cavity is CCR2 dependent.** Use of an *in vitro* under-agarose chemotaxis assay revealed that HdAg was not directly chemotactic for bone marrow-derived macrophages over an 18-h period (see Fig. S4 in the supplemental material). Gating on the F4/80$^+$ Gr-1$^{lo}$ cells retrieved from the peritoneal cavity 24 h after HdAg treatment revealed that the majority of these cells were CCR2$^+$ and CXCR2$^-$ (see Fig. S5 in the supplemental material). Significantly fewer PECs were retrieved from HdAg-treated CCR2$^{-/-}$ mice (Fig. 6A) with reduced numbers of F4/80$^+$ Gr-1$^{lo}$ cells (Fig. 6B). Thus, CCR2 and PD-L1 are signature markers for these M$^{(HdAg)}$ cells, with the former required for recruitment into the peritoneal cavity.

**Adoptive transfer of F4/80$^+$ Gr-1$^{lo}$ cells mobilized in response to HdAg inhibits DSS-induced colitis.** The preceding data show that the HdAg promotes the occurrence of CCR2$^+$ PD-L1$^+$ F4/80$^+$ Gr-1$^{lo}$ LyC6$^+$ monocytic cells, yet a role for these cells, if any, in the suppression of colitis was unknown. Thus, F4/80$^+$ Gr-1$^{lo}$ monocytic cells were sorted from the peritoneal cavity of mice treated 24 h previously with HdAg (1 mg), their expression of LyC6 was confirmed, and then, in turn, the cells were administered to mice beginning on day 2 of the DSS regimen (Fig. 7A). With the exception of no improvement in body weight (Fig. 7B), the transferred M$^{(HdAg)}$ cells significantly reduced the severity of DSS-induced disease as gauged by colon length, macroscopic disease score, and histopathology (Fig. 7C to F). Analysis of splenic cytokines revealed that the suppression of colitis correlated with reduced IL-17 and TNF-α production and increased synthesis of IL-4 and IL-10 (Fig. 7G), analogous to the pattern observed following delivery of *H. diminuta* antigen into the peritoneal cavity and DNBS challenge (Fig. 2).

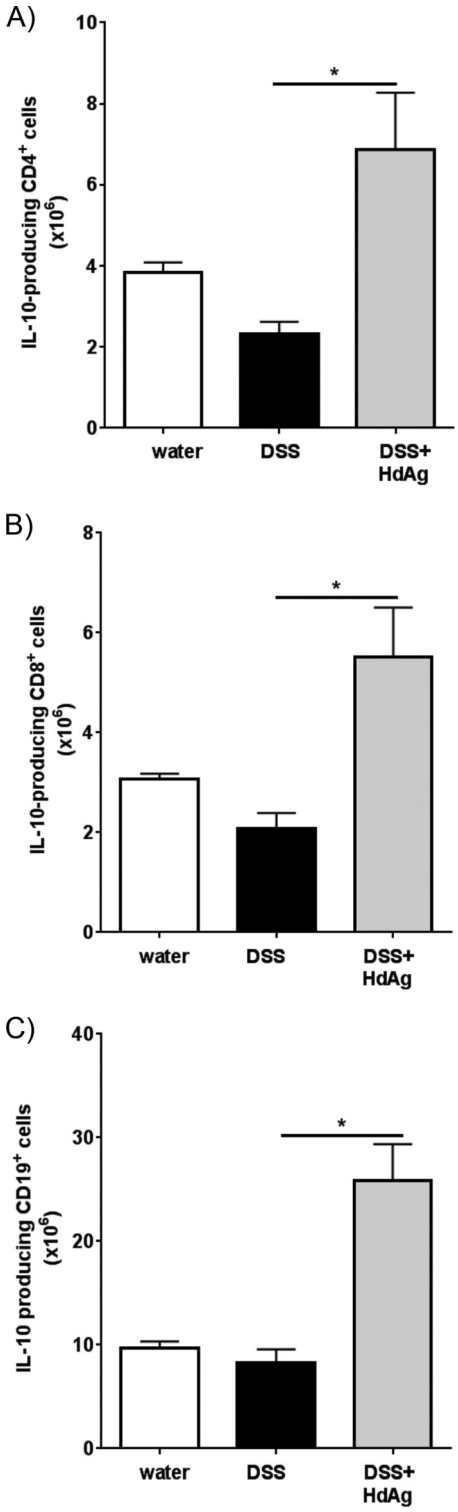


**FIG 3** IL-10 is increased in splenocytes from HdAg-treated mice. Total spleen cells ($5 \times 10^6$) from the indicated experimental groups (Fig. 1A) were stimulated with anti-CD3/anti-CD28 antibodies for 96 h, and intracellular IL-10 and surface CD4, CD8, and CD19 staining was performed, followed by flow cytometry. The numbers of IL-10-producing CD4 (A), CD8 (B), and CD19 (C) cells were calculated based on percentage of positivity and total spleen cell counts. Data are means ± SEM from $n = 5$. *, $P < 0.05$ compared to the DSS group.

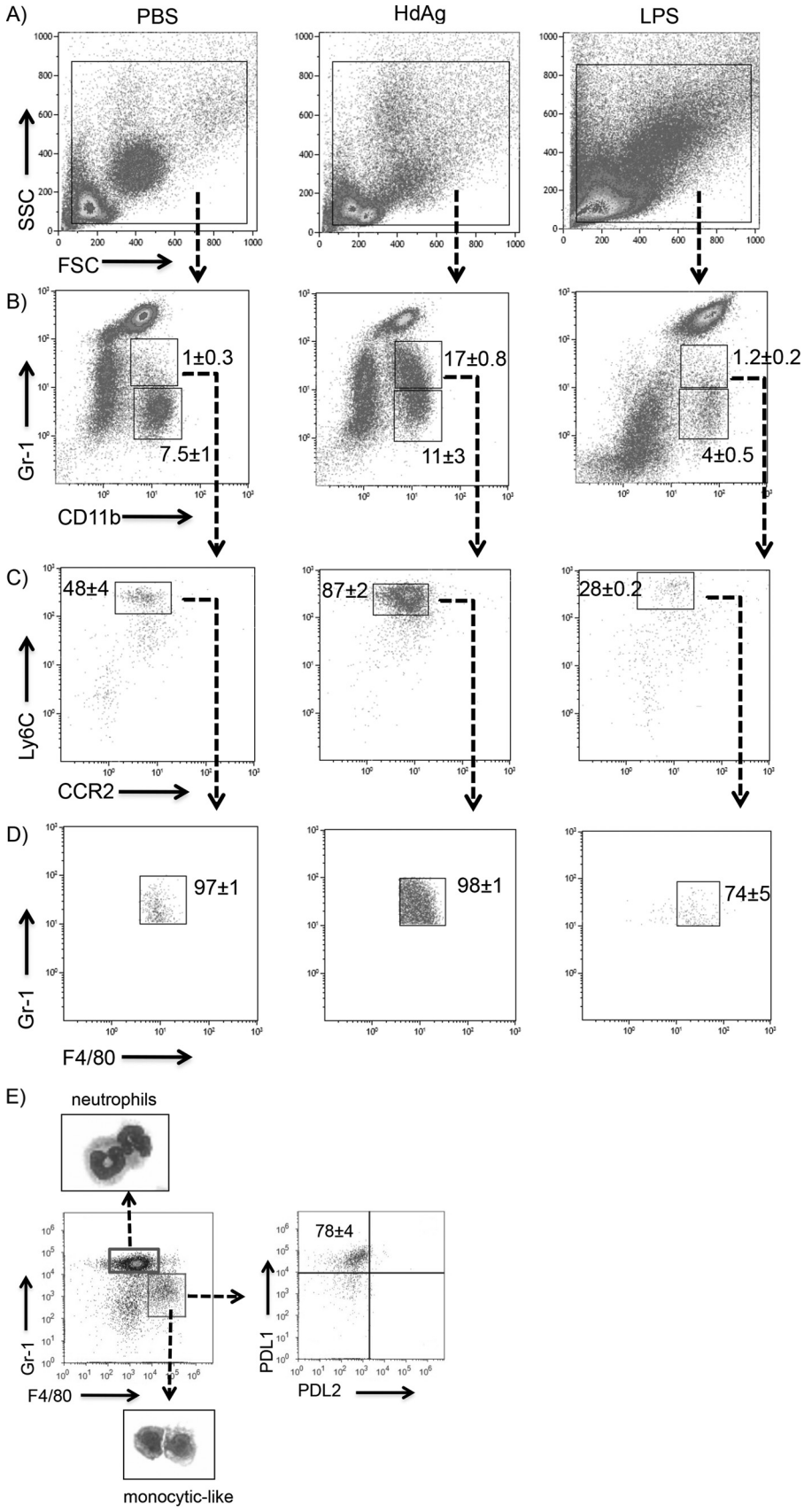


**FIG 4** *H. diminuta* antigens recruit monocytic-like cells. Peritoneal cells were obtained 24 h after HdAg (1 mg), LPS (1 μg), or PBS injection. Panel A demonstrates changes in cell distribution based on forward and side scatter (FSC and SSC, respectively) parameters. Distinct populations of $CD11b^+$ $Gr\text{-}1^-$ or $Gr\text{-}1^{hi}$ and $Gr\text{-}1^{lo}$ cells are apparent (B), with a significant increase in $CD11b^+$ $Gr\text{-}1^{lo}$ infiltrating cells in response to HdAg. Characterization of Ly6C and CCR2 (C) and F4/80 (D) defined this population as $CD11b^+$ $Gr\text{-}1^{lo}$ $Ly6C^+$ $F4/80^+$ monocyte-like cells. (E) H&E staining of the sorted cells revealed a polymorphonuclear morphology in the $F4/80^-$ $Gr\text{-}1^{hi}$ cells consistent with neutrophils, whereas the $F4/80^+$ $Gr\text{-}1^{lo}$ cells were of monocyte/macrophage appearance and were predominantly PD-$L1^+$ PD-$L2^-$ ($n = 4$ to 6).

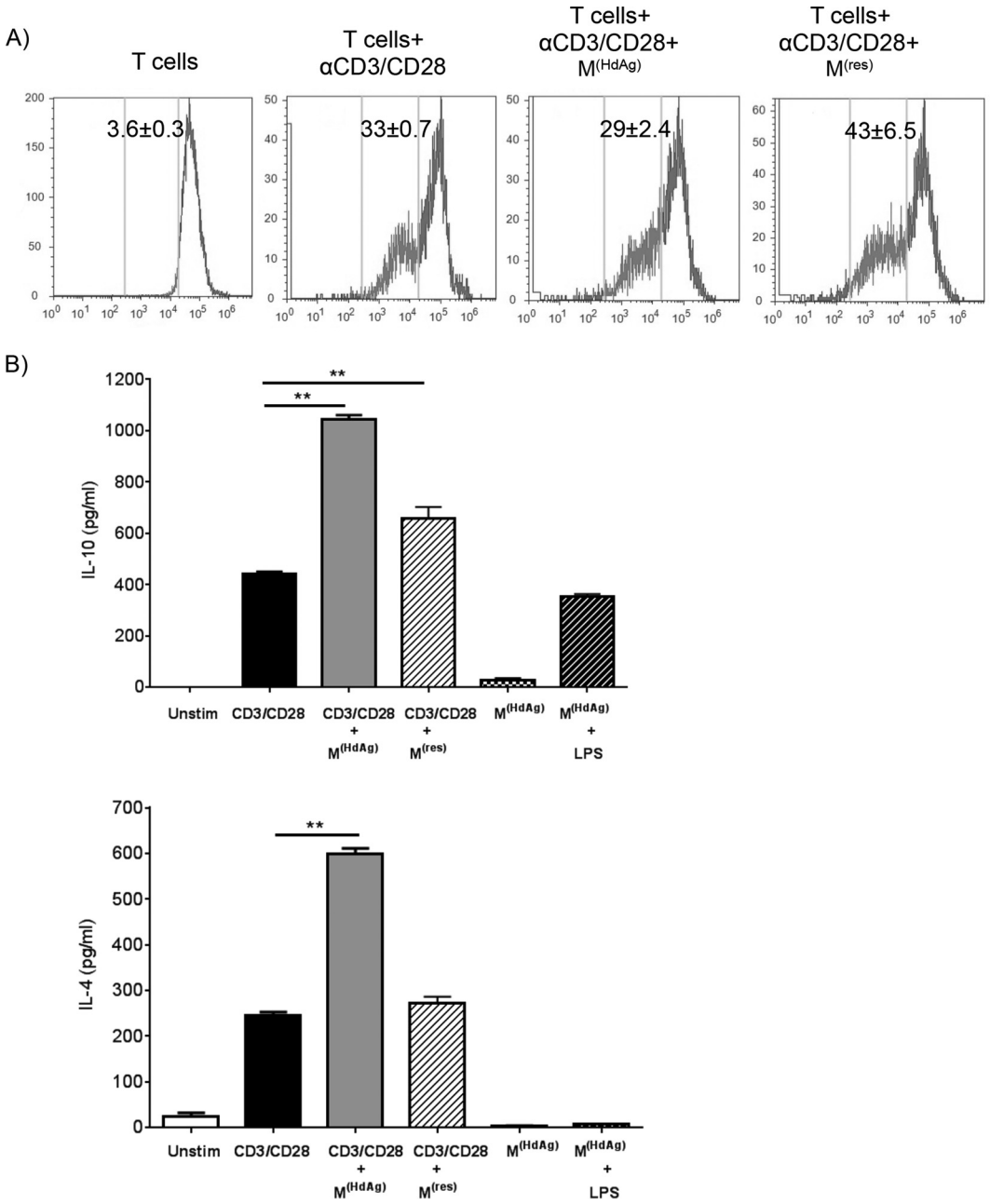


**FIG 5** HdAg-recruited monocytic cells induce IL-10 and IL-4 in splenic $CD4^+$ T cell cocultures. Coculture of sorted $F4/80^+$ $Gr\text{-}1^{lo}$ cells ($2.5 \times 10^5$) from HdAg-treated [1 mg i.p., 24 h; $M^{(HdAg)}$] mice with anti-CD3+anti-CD28 activated splenic $CD4^+$ T cells ($1 \times 10^6$) did not affect T cell proliferation (gauged by CSFE dilution) (A) but significantly increased IL-10 and IL-4 (measured at 96 h) compared to coculture with $F4/80^+$ $Gr\text{-}1^-$ peritoneal cells from PBS-injected mice [$M^{(res)}$] (B). Unstim, unstimulated. Data are means ± SEM from 2 independent experiments with $n = 6$ mice. **, $P < 0.01$ compared to the indicated group. "CD3/CD28" indicates T cell activation conditions. The LPS concentration was 1 μg/ml.

## DISCUSSION

Helminth parasites have evolved to elude or suppress their hosts' immune response, and this implies that they are a source of immunoregulatory molecules. Many studies show that tissue extracts or E/S products from helminths can suppress mammalian immune cell activation *in vitro* (29–32) and ameliorate the severity of inflammatory disease in murine model systems (14, 18, 19, 29, 30, 33, 34). The demonstration herein that systemic administration of a crude extract of adult *H. diminuta* parasites dose-dependently inhibits DSS-induced colitis adds to awareness of the ability of helminth-derived molecules to suppress intestinal inflammation (19, 21, 35).

Mice infected with *H. diminuta* are protected from colitis induced by intrarectal administration of DNBS (10): daily doses of HdAg also block this disease (21). However, infection with *H. diminuta* provided only a modest benefit in DSS-induced colitis (20) and exaggerated oxazolone-induced colitis (36). Infection with a viable helminth represents a very different challenge to the

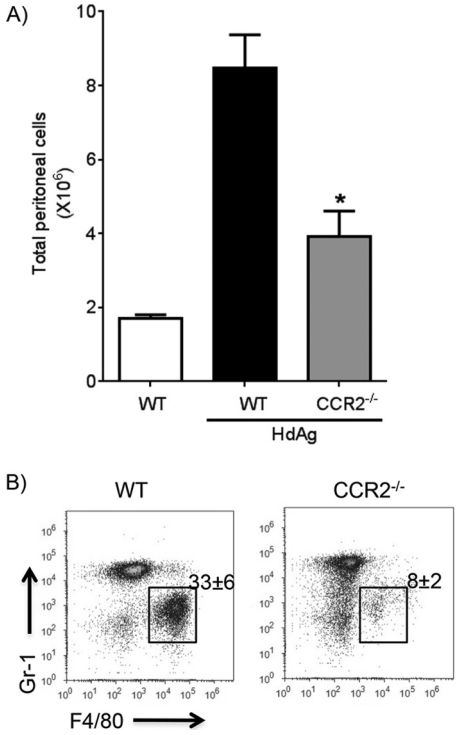


**FIG 6** CCR2 is required for HdAg-recruited monocytic cells. Wild-type (WT) and CCR2$^{-/-}$ mice received i.p. injection of HdAg (1 mg) or PBS, and 24 h later, peritoneal exudate cells (PECs) were harvested and counted (A). Infiltration of F4/80$^{+}$ Gr-1$^{lo}$ cells was significantly reduced in the CCR2$^{-/-}$ mice compared to WT mice (B). Data are means ± SEM from 2 independent experiments with $n = 8$ mice. * and #, $P < 0.05$ compared to PECs from untreated and HdAg-treated WT mice, respectively.

immune system compared to systemic delivery of antigens. Testing the possibility that HdAg would suppress DSS-induced colitis revealed that intraperitoneal injection of HdAg significantly reduced the severity of disease.

Exposure to HdAg could elicit a variety of local or systemic events to suppress inflammation: for example, HdAg can suppress LPS-induced TNF-α from murine macrophages (21), and bone marrow-derived dendritic cells pulsed with HdAg can ameliorate colitis (37). The peritoneal cavity is emerging as a site of immune regulation (38) and a reservoir of tissue-repairing macrophages (39). Amelioration of trinitrobenzene sulfonic acid (TNBS)-induced colitis was greater with i.p. versus intravenous delivery of mesenchymal stem cells (40), and the mesenchymal cells blocked colitis without the need to localize to the colon (41). Hoeksema et al. showed that *Trichuris suis* soluble products evoke an M2-type macrophage via epigenetic changes (42). Also, macrophages cotreated with *Ascaris suum* E/S products and immune serum displayed enhanced wound-healing activity in an *in vitro* model (62). Thus, we speculate that monocytes recruited into the peritoneal cavity are converted into immunoregulatory monocyte-like cells by exposure to the HdAg and/or released host factors, and that these cells represent another mechanism by which HdAg treatment could ameliorate colitis. Whether these cells trigger events in the peritoneal cavity or if they need to traffic to mesenteric lymph nodes (MLNs), spleen, or the colon to ameliorate colitis remains to be determined.

Following HdAg injection, there was an early (by 4 h) accumulation of F4/80$^{-}$ Gr1$^{+}$cells (predominantly neutrophils) in the peritoneal cavity that was replaced by an F4/80$^{+}$ Gr-1$^{lo}$ LyC6$^{+}$ cells, whose recruitment was critically dependent on CCR2 expression. (HdAg by itself was not chemotactic for monocytes.) These cells may represent a unique myeloid-derived suppressor cell (MDSC) phenotype (43, 44). A requirement for CCR2 has been described for the recruitment/expansion of MDSCs that can exert either beneficial (e.g., suppression of experimental arthritis) or detrimental (e.g., kidney tumor progression) health effects (45, 46). Antigens derived from flatworms do recruit myeloid cells, and these cells blocked *in vitro* T cell activation; however, neither the phenotype nor *in vivo* functions of these cells were reported (27, 28, 47). The HdAg-evoked monocytic cells lacked CXCR2, implying an immunosuppressive character, since CXCR2$^{+}$ MDSCs have been implicated in colitis-associated cancer (48) and in *Helicobacter pylori*-induced gastritis (49), and CXCR2$^{+}$ monocytic cells have been implicated in the development of atherosclerosis (50).

The M$^{(HdAg)}$ cells did not suppress T cell proliferation, a classic feature of MDSC cells (43), but did induce increased IL-10 (and IL-4) from cocultures of splenic CD4$^{+}$ T cells (anti-CD3/CD28 activated). Mobilization of IL-10 is a common strategy in the suppression of inflammatory disease. Indeed, the inhibition of colitis evoked by infection with parasitic helminths, including *H. diminuta*, or treatment with worm antigen is often accompanied by increased IL-10 (10, 32, 51). Ligation of the programmed death 1 (PD-1) receptor is an important checkpoint in T cell proliferation (52). Infection with helminths or exposure to worm antigen can provoke increased expression of PD-L1 and PD-L2 on myeloid cells (53, 54), and functional studies indicate that suppression of T cell activity in chronic helminth infections is mediated, at least in part, by PD-L2$^{+}$ macrophages (54, 55). In comparison, the M$^{(HdAg)}$ cells were PD-L1$^{+}$ and PD-L2$^{-}$ (described below).

A role for the HdAg-recruited monocytic cells in the amelioration of colitis was unknown and could have been irrelevant given the number of anticolitic events that could be evoked by systemic delivery of the antigen. When delivered into the peritoneal cavity in a treatment strategy (i.e., 2 days after exposure to DSS), the F4/80$^{+}$ Gr-1$^{lo}$ Ly6C$^{+}$ cells significantly reduced the severity of DSS-induced colitis. Preliminary data suggest that the suppression of colitis is PD-L1 independent (personal observation using neutralizing anti-PDL-1 antibodies), although additional studies with cells lacking PD-L1 will be required to confirm or refute this postulate.

In accordance with the data on HdAg-evoked F4/80$^{+}$ Gr-1$^{lo}$ Ly6C$^{+}$ monocytic cells, increases in CD11b$^{+}$ Gr1$^{+}$ MDSCs have been found in the blood and spleen of protein tyrosine phosphatase 1B$^{-/-}$ mice treated with DSS and in the spleen of mice treated with reversatrol plus DSS. While these findings correlated with less DSS-induced colitis, neither study reported cause-effect experiments (56, 57). Also, increases in CD11b$^{+}$ Gr-1$^{+}$ MDSCs have been found in the spleen and lamina propria of mice with TNBS-induced colitis, and administration of these cells isolated from the spleen inhibited colitis (58). Administration of splenic CD11b$^{+}$ Gr-1$^{+}$ cells at the end of a 5-day 2% DSS regimen in C57BL/6 mice hastened the recovery from colitis (59). With both of these studies, it is unclear if the anticolitic effect was due to Ly6C$^{+}$ or Ly6G$^{+}$ MDSCs. In addition, circulating immunosuppressive Gr-1$^{+}$ monocytes have been shown to restrict experimental allergic encephalitis in mice (32, 60).

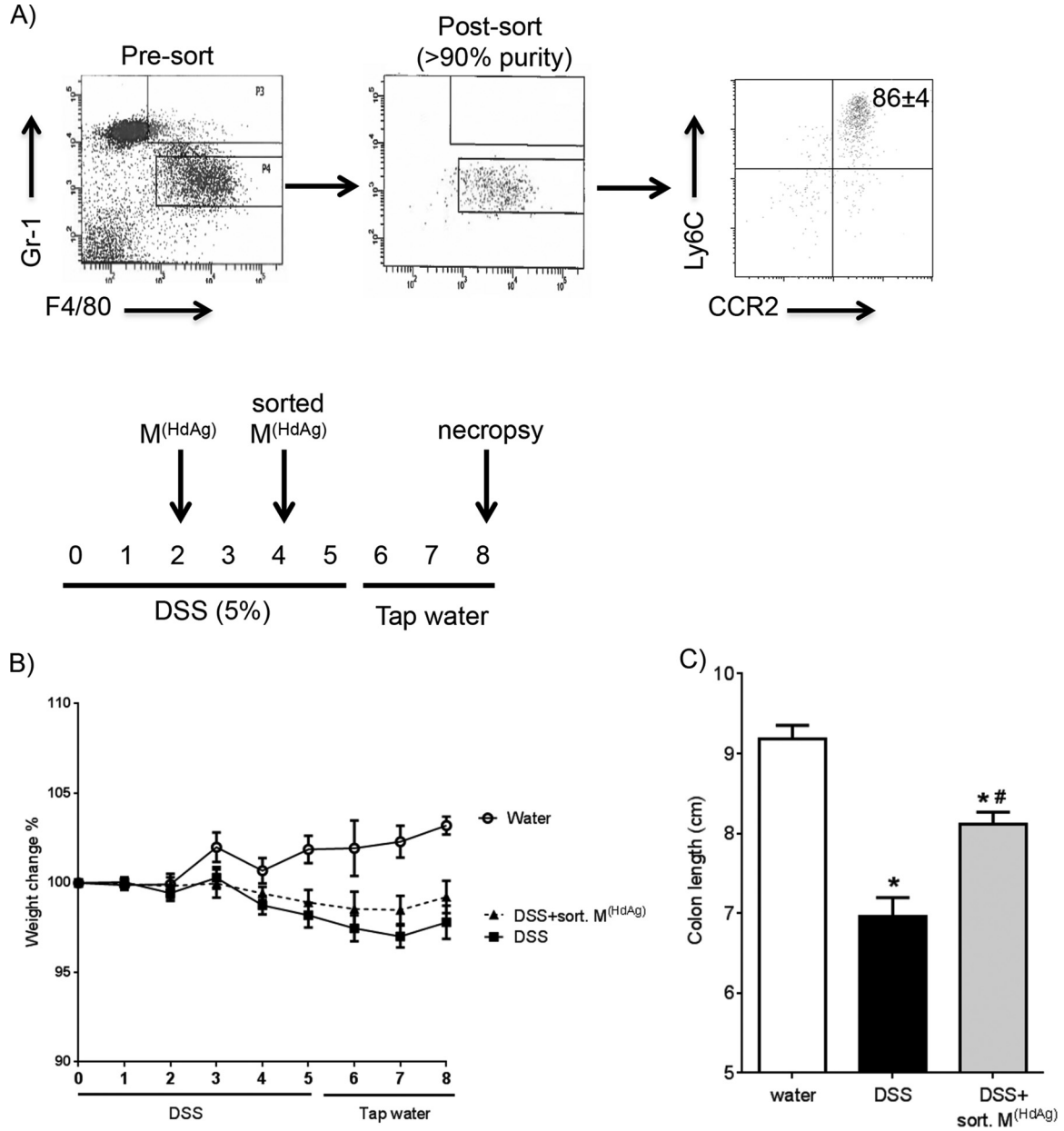


**FIG 7** Transfer of monocytic cells recruited by HdAg attenuates DSS-induced colitis. Mice given HdAg (1 mg i.p.) were necropsied 24 h later, and peritoneal cells were stained as indicated in Materials and Methods. Thereafter, F4/80$^+$ Gr1$^{lo}$ Ly6c$^+$ cells were sorted and collected and used as shown in the experimental paradigm in panel A ($1 \times 10^6$ cells i.p.). On necropsy, severity of colitis was determined by change in body weight (B), colon length (C and D), disease activity score (E), and histopathology (F). In panel F, representative H&E-stained sections of midcolon are shown (scale bars, 100 μm). L, lumen; M, mucosa. Data are means ± SEM from 3 experiments with $n = 9$ (controls) or 12 (all other groups) mice. Panel G shows cytokine production from stimulated spleen cells (see Materials and Methods) ($n = 4$). * and #, $P < 0.05$ compared to mice treated with water only and mice treated with DSS, respectively.

It is not known if these various monocytic myeloid populations are substantially or subtly different or if they block disease via common or divergent mechanisms. The precise anticolitic mechanism of action of all the aforementioned myeloid cells, including putative *in vivo* interaction(s) with other immune and stromal cells, demands focused research efforts. Nevertheless, a body of evidence is emerging demonstrating that monocytic, MDSCs, or MDSC-like cells can suppress colitis in murine models, begging the question of whether similar phenotypes in humans could be used to treat IBD; however, the possibility exists that this therapy could predispose the patient to infection or carcinogenesis.

In summary, injection of an extract of adult *H. diminuta* dose-dependently inhibited DSS-induced colitis and was accompanied by recruitment of CCR2$^+$ PD-L1$^+$ F4/80$^+$ Gr-1$^{lo}$ Ly6C$^+$ monocytic cells. The adoptive transfer of these M$^{(HdAg)}$ cells inhibited DSS-induced colitis, demonstrating a novel aspect by which helminth-derived molecules could suppress intestinal inflammation. It is apparent that infection with viable helminth parasites, treat-

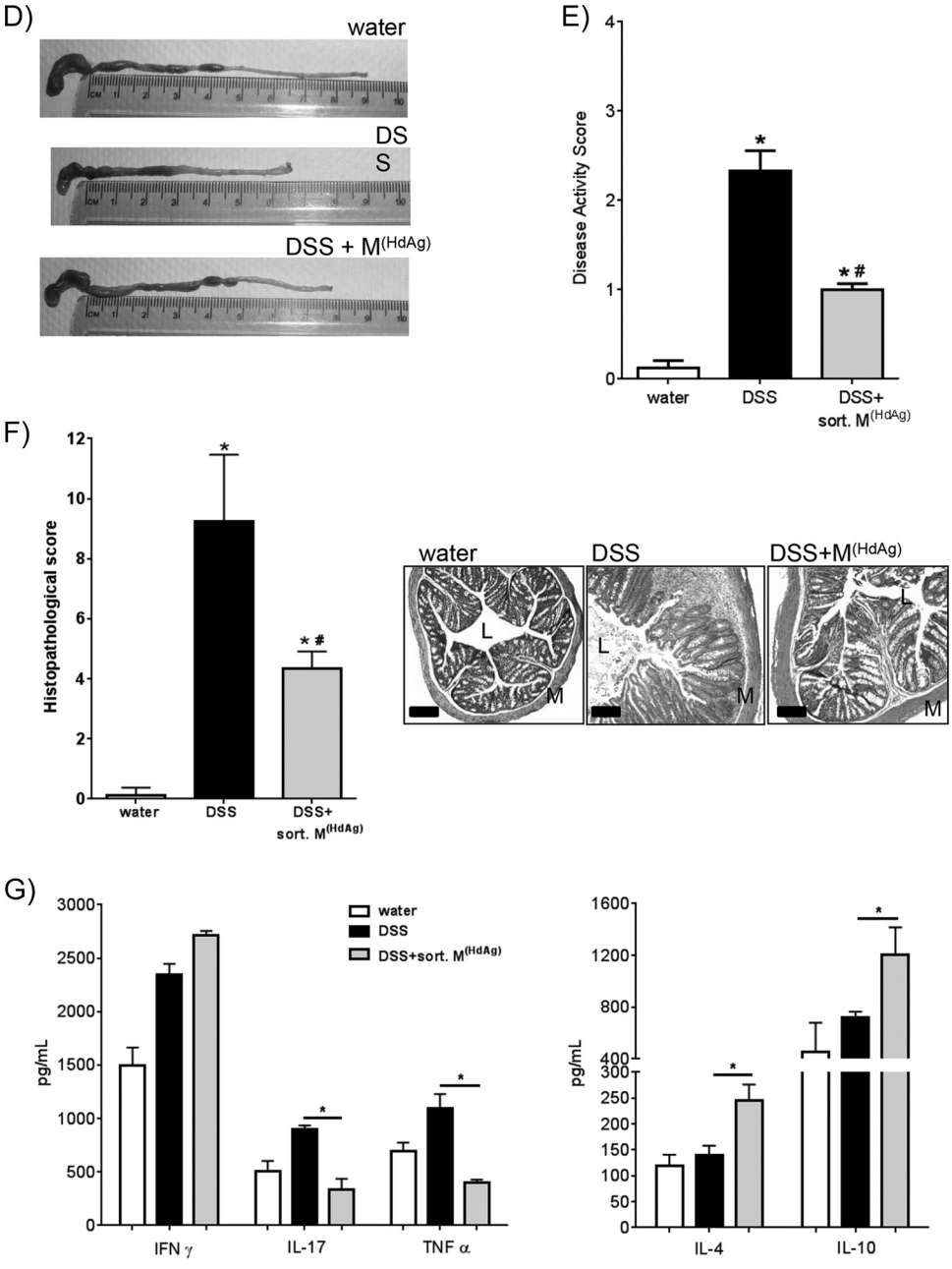


FIG 7 continued

ment with a variety of helminth extracts/antigens, and delivery of either immune cells retrieved from infected individuals or immune cells educated *in vitro* with helminth antigens all have the capacity to block inflammatory disease (61). The challenge is to define the immunological regulation associated with each of these approaches to suppress inflammation and determine the optimal means to translate this knowledge to the treatment of inflammatory disease.

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## REFERENCES

1. **McSorley HJ, Hewitson JP, Maizels RM.** 2013. Immunomodulation by helminth parasites: defining mechanisms and mediators. Int J Parasitol **43:**301–310. http://dx.doi.org/10.1016/j.ijpara.2012.11.011.
2. **La Flamme AC, Ruddenklau K, Backstrom BT.** 2003. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. Infect Immun **71:**4996–5004. http://dx.doi.org/10.1128/IAI.71.9.4996-5004.2003.
3. **Wu Z, Nagano I, Asano K, Takahashi Y.** 2010. Infection of non-encapsulated species of Trichinella ameliorates experimental autoimmune encephalomyelitis involving suppression of Th17 and Th1 response. Parasitol Res **107:**1173–1188. http://dx.doi.org/10.1007/s00436-010-1985-9.
4. **Reyes JL, Espinoza-Jimenez AF, Gonzalez MI, Verdin L, Terrazas LI.** 2011. *Taenia crassiceps* infection abrogates experimental autoimmune encephalomyelitis. Cell Immunol **267:**77–87. http://dx.doi.org/10.1016/j.cellimm.2010.11.006.
5. **Salinas-Carmona MC, de la Cruz-Galicia G, Perez-Rivera I, Solis-Soto JM, Segoviano-Ramirez JC, Vazquez AV, Garza MA.** 2009. Spontaneous arthritis in MRL/lpr mice is aggravated by *Staphylococcus aureus* and ameliorated by *Nippostrongylus brasiliensis* infections. Autoimmunity **42:**25–32. http://dx.doi.org/10.1080/08916930802228290.
6. **Osada Y, Shimizu S, Kumagai T, Yamada S, Kanazawa T.** 2009. *Schistosoma mansoni* infection reduces severity of collagen-induced arthritis via down-regulation of pro-inflammatory mediators. Int J Parasitol **39:**457–464. http://dx.doi.org/10.1016/j.ijpara.2008.08.007.
7. **Shi M, Wang A, Prescott D, Waterhouse CC, Zhang S, McDougall JJ, Sharkey KA, McKay DM.** 2011. Infection with an intestinal helminth parasite reduces Freund's complete adjuvant-induced monoarthritis in mice. Arthritis Rheum **63:**434–444. http://dx.doi.org/10.1002/art.30098.
8. **Khan WI, Blennerhasset PA, Varghese AK, Chowdhury SK, Omsted P, Deng Y, Collins SM.** 2002. Intestinal nematode infection ameliorates experimental colitis in mice. Infect Immun **70:**5931–5937. http://dx.doi.org/10.1128/IAI.70.11.5931-5937.2002.
9. **Elliott DE, Setiawan T, Metwali A, Blum A, Urban JF, Jr, Weinstock JV.** 2004. *Heligmosomoides polygyrus* inhibits established colitis in IL-10-deficient mice. Eur J Immunol **34:**2690–2698. http://dx.doi.org/10.1002/eji.200324833.
10. **Hunter MM, Wang A, Hirota CL, McKay DM.** 2005. Neutralizing anti-IL-10 antibody blocks the protective effect of tapeworm infection in a murine model of chemically induced colitis. J Immunol **174:**7368–7375. http://dx.doi.org/10.4049/jimmunol.174.11.7368.
11. **Smits HH, Hammad H, van Nimwegen M, Soullie T, Willart MA, Lievers E, Kadouch J, Kool M, Kos-van Oosterhoud J, Deelder AM, Lambrecht BN, Yazdanbakhsh M.** 2007. Protective effect of *Schistosoma mansoni* infection on allergic airway inflammation depends on the intensity and chronicity of infection. J Allergy Clin Immunol **120:**932–940. http://dx.doi.org/10.1016/j.jaci.2007.06.009.
12. **Aranzamendi C, de Bruin A, Kuiper R, Boog CJ, van Eden W, Rutten V, Pinelli E.** 2013. Protection against allergic airway inflammation during the chronic and acute phases of *Trichinella spiralis* infection. Clin Exp Allergy **43:**103–115. http://dx.doi.org/10.1111/cea.12042.
13. **Zheng X, Hu X, Zhou G, Lu Z, Qiu W, Bao J, Dai Y.** 2008. Soluble egg antigen from *Schistosoma japonicum* modulates the progression of chronic progressive experimental autoimmune encephalomyelitis via Th2-shift response. J Neuroimmunol **194:**107–114. http://dx.doi.org/10.1016/j.jneuroim.2007.12.001.
14. **Ferreira I, Smyth D, Gaze S, Aziz A, Giacomin P, Ruyssers N, Artis D, Laha T, Navarro S, Loukas A, McSorley HJ.** 2013. Hookworm excretory/secretory products induce interleukin-4 (IL-4)$^+$ IL-10$^+$ CD4$^+$ T cell responses and suppress pathology in a mouse model of colitis. Infect Immun **81:**2104–2111. http://dx.doi.org/10.1128/IAI.00563-12.
15. **McSorley HJ, Blair NF, Robertson E, Maizels RM.** 2015. Suppression of OVA-alum induced allergy by Heligmosomoides polygyrus products is MyD88-, TRIF-, regulatory T- and B cell-independent, but is associated with reduced innate lymphoid cell activation. Exp Parasitol **158:**8–17. http://dx.doi.org/10.1016/j.exppara.2015.02.009.
16. **Elliott DE, Urban JJ, Argo CK, Weinstock JV.** 2000. Does the failure to acquire helminthic parasites predispose to Crohn's disease? FASEB J **14:**1848–1855. http://dx.doi.org/10.1096/fj.99-0885hyp.
17. **Smith P, Mangan NE, Walsh CM, Fallon RE, McKenzie AN, van Rooijen N, Fallon PG.** 2007. Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism. J Immunol **178:**4557–4566. http://dx.doi.org/10.4049/jimmunol.178.7.4557.
18. **Yang X, Yang Y, Wang Y, Zhan B, Gu Y, Cheng Y, Zhu X.** 2014. Excretory/secretory products from *Trichinella spiralis* adult worms ameliorate DSS-induced colitis in mice. PLoS One **9:**e96454. http://dx.doi.org/10.1371/journal.pone.0096454.
19. **Heylen M, Ruyssers NE, Nullens S, Schramm G, Pelckmans PA, Moreels TG, De Man JG, De Winter BY.** 2015. Treatment with egg antigens of *Schistosoma mansoni* ameliorates experimental colitis in mice through a colonic T-cell-dependent mechanism. Inflamm Bowel Dis **21:**48–59. http://dx.doi.org/10.1097/MIB.0000000000000246.
20. **Reardon C, Sanchez A, Hogaboam CM, McKay DM.** 2001. Tapeworm infection reduces epithelial ion transport abnormalities in murine dextran sulfate sodium-induced colitis. Infect Immun **69:**4417–4423. http://dx.doi.org/10.1128/IAI.69.7.4417-4423.2001.
21. **Johnston MJ, Wang A, Catarino ME, Ball L, Phan VC, MacDonald JA, McKay DM.** 2010. Extracts of the rat tapeworm, Hymenolepis diminuta, suppress macrophage activation in vitro and alleviate chemically induced colitis in mice. Infect Immun **78:**1364–1375. http://dx.doi.org/10.1128/IAI.01349-08.
22. **Reyes JL, Wang A, Fernando MR, Graepel R, Leung G, van Rooijen N, Sigvardsson M, McKay DM.** 2015. Splenic B cells from *Hymenolepis diminuta*-infected mice ameliorate colitis independent of T cells and via cooperation with macrophages. J Immunol **194:**364–378. http://dx.doi.org/10.4049/jimmunol.1400738.
23. **Fernando MR, Reyes JL, Iannuzzi J, Leung G, McKay DM.** 2014. The pro-inflammatory cytokine, interleukin-6, enhances the polarization of alternatively activated macrophages. PLoS One **9:**e94188. http://dx.doi.org/10.1371/journal.pone.0094188.
24. **Heit B, Kubes P.** 2003. Measuring chemotaxis and chemokinesis: the under-agarose cell migration assay. Sci STKE **2003:**PL5.
25. **Shouval DS, Ouahed J, Biswas A, Goettel JA, Horwitz BH, Klein C, Muise AM, Snapper SB.** 2014. Interleukin 10 receptor signaling: master regulator of intestinal mucosal homeostasis in mice and humans. Adv Immunol **122:**177–210. http://dx.doi.org/10.1016/B978-0-12-800267-4.00005-5.
26. **Engelhardt KR, Grimbacher B.** 2014. IL-10 in humans: lessons from the gut, IL-10/IL-10 receptor deficiencies, and IL-10 polymorphisms. Curr Top Microbiol Immunol **380:**1–18. http://dx.doi.org/10.1007/978-3-662-43492-5_1.
27. **Atochina O, Daly-Engel T, Piskorska D, McGuire E, Harn DA.** 2001. A schistosome-expressed immunomodulatory glycoconjugate expands peritoneal Gr1$^+$ macrophages that suppress naive CD4$^+$ T cell proliferation via an IFN-γ and nitric oxide-dependent mechanism. J Immunol **167:**4293–4302. http://dx.doi.org/10.4049/jimmunol.167.8.4293.
28. **Gomez-Garcia L, Lopez-Marin LM, Saavedra R, Reyes JL, Rodriguez-Sosa M, Terrazas LI.** 2005. Intact glycans from cestode antigens are involved in innate activation of myeloid suppressor cells. Parasite Immunol **27:**395–405. http://dx.doi.org/10.1111/j.1365-3024.2005.00790.x.
29. **Terrazas CA, Sanchez-Munoz F, Mejia-Dominguez AM, Amezcua-Guerra LM, Terrazas LI, Bojalil R, Gomez-Garcia L.** 2011. Cestode antigens induce a tolerogenic-like phenotype and inhibit LPS inflammatory responses in human dendritic cells. Int J Biol Sci **7:**1391–1400.
30. **Langelaar M, Aranzamendi C, Franssen F, Van Der Giessen J, Rutten V, van der Ley P, Pinelli E.** 2009. Suppression of dendritic cell maturation

by *Trichinella spiralis* excretory/secretory products. Parasite Immunol **31:** 641–645. http://dx.doi.org/10.1111/j.1365-3024.2009.01136.x.

31. **Zaccone P, Burton O, Miller N, Jones FM, Dunne DW, Cooke A.** 2009. *Schistosoma mansoni* egg antigens induce Treg that participate in diabetes prevention in NOD mice. Eur J Immunol **39:**1098–1107. http://dx.doi.org/10.1002/eji.200838871.
32. **Ziegler T, Rausch S, Steinfelder S, Klotz C, Hepworth MR, Kuhl AA, Burda PC, Lucius R, Hartmann S.** 2015. A novel regulatory macrophage induced by a helminth molecule instructs IL-10 in $CD4^+$ T cells and protects against mucosal inflammation. J Immunol **194:**1555–1564. http://dx.doi.org/10.4049/jimmunol.1401217.
33. **Martin I, Caban-Hernandez K, Figueroa-Santiago O, Espino AM.** 2015. *Fasciola hepatica* fatty acid binding protein inhibits TLR4 activation and suppresses the inflammatory cytokines induced by lipopolysaccharide *in vitro* and *in vivo*. J Immunol **194:**3924–3936. http://dx.doi.org/10.4049/jimmunol.1401182.
34. **Tang GX, Zhou HJ, Xu JW, Xu JM, Ji MJ, Wu HW, Wu GL.** 2012. *Schistosoma japonicum* soluble egg antigens attenuate IFN-γ-induced MHC class II expression in RAW 264.7 macrophages. PLoS One **7:**e49234. http://dx.doi.org/10.1371/journal.pone.0049234.
35. **Du L, Tang H, Ma Z, Xu J, Gao W, Chen J, Gan W, Zhang Z, Yu X, Zhou X, Hu X.** 2011. The protective effect of the recombinant 53-kDa protein of *Trichinella spiralis* on experimental colitis in mice. Dig Dis Sci **56:**2810–2817. http://dx.doi.org/10.1007/s10620-011-1689-8.
36. **Wang A, Fernando M, Leung G, Phan V, Smyth D, McKay DM.** 2010. Exacerbation of oxazolone colitis by infection with the helminth *Hymenolepis diminuta*: involvement of IL-5 and eosinophils. Am J Pathol **177:**2850–2859. http://dx.doi.org/10.2353/ajpath.2010.100537.
37. **Matisz CE, Leung G, Reyes JL, Wang A, Sharkey KA, McKay DM.** 2015. Adoptive transfer of helminth antigen-pulsed dendritic cells protects against the development of experimental colitis in mice. Eur J Immunol **45:**3126–3139. http://dx.doi.org/10.1002/eji.201545579.
38. **Okabe Y, Medzhitov R.** 2014. Tissue-specific signals control reversible program of localization and functional polarization of macrophages. Cell **157:**832–844. http://dx.doi.org/10.1016/j.cell.2014.04.016.
39. **Wang J, Kubes P.** 2016. A reservoir of mature cavity macrophages that can rapidly invade visceral organs to affect tissue repair. Cell **165:**668–678. http://dx.doi.org/10.1016/j.cell.2016.03.009.
40. **Castelo-Branco MT, Soares ID, Lopes DV, Buongusto F, Martinusso CA, do Rosario A, Jr, Souza SA, Gutfilen B, Fonseca LM, Elia C, Madi K, Schanaider A, Rossi MI, Souza HS.** 2012. Intraperitoneal but not intravenous cryopreserved mesenchymal stromal cells home to the inflamed colon and ameliorate experimental colitis. PLoS One **7:**e33360. http://dx.doi.org/10.1371/journal.pone.0033360.
41. **Sala E, Genua M, Petti L, Anselmo A, Arena V, Cibella J, Zanotti L, D'Alessio S, Scaldaferri F, Luca G, Arato I, Calafiore R, Sgambato A, Rutella S, Locati M, Danese S, Vetrano S.** 2015. Mesenchymal stem cells reduce colitis in mice via release of TSG6, independently of their localization to the intestine. Gastroenterology **149:**163–176. http://dx.doi.org/10.1053/j.gastro.2015.03.013.
42. **Hoeksema MA, Laan LC, Postma JJ, Cummings RD, de Winther MP, Dijkstra CD, van Die I, Kooij G.** 2016. Treatment with *Trichuris suis* soluble products during monocyte-to-macrophage differentiation reduces inflammatory responses through epigenetic remodeling. FASEB J **30:**2826–2836. http://dx.doi.org/10.1096/fj.201600343R.
43. **Talmadge JE, Gabrilovich DI.** 2013. History of myeloid-derived suppressor cells. Nat Rev Cancer **13:**739–752. http://dx.doi.org/10.1038/nrc3581.
44. **Van Ginderachter JA, Beschin A, De Baetselier P, Raes G.** 2010. Myeloid-derived suppressor cells in parasitic infections. Eur J Immunol **40:** 2976–2985. http://dx.doi.org/10.1002/eji.201040911.
45. **Hale M, Itani F, Buchta CM, Wald G, Bing M, Norian LA.** 2015. Obesity triggers enhanced MDSC accumulation in murine renal tumors via elevated local production of CCL2. PLoS One **10:**e0118784. http://dx.doi.org/10.1371/journal.pone.0118784.
46. **Crook KR, Jin M, Weeks MF, Rampersad RR, Baldi RM, Glekas AS, Shen Y, Esserman DA, Little P, Schwartz TA, Liu P.** 2015. Myeloid-derived suppressor cells regulate T cell and B cell responses during autoimmune disease. J Leukoc Biol **97:**573–582. http://dx.doi.org/10.1189/jlb.4A0314-139R.
47. **Terrazas LI, Walsh KL, Piskorska D, McGuire E, Harn DA, Jr.** 2001. The schistosome oligosaccharide lacto-N-neotetraose expands $Gr1^+$ cells that secrete anti-inflammatory cytokines and inhibit proliferation of naive $CD4^+$ cells: a potential mechanism for immune polarization in helminth infections. J Immunol **167:**5294–5303. http://dx.doi.org/10.4049/jimmunol.167.9.5294.
48. **Katoh H, Wang D, Daikoku T, Sun H, Dey SK, Dubois RN.** 2013. CXCR2-expressing myeloid-derived suppressor cells are essential to promote colitis-associated tumorigenesis. Cancer Cell **24:**631–644. http://dx.doi.org/10.1016/j.ccr.2013.10.009.
49. **Zhuang Y, Cheng P, Liu XF, Peng LS, Li BS, Wang TT, Chen N, Li WH, Shi Y, Chen W, Pang KC, Zeng M, Mao XH, Yang SM, Guo H, Guo G, Liu T, Zuo QF, Yang HJ, Yang LY, Mao FY, Lv YP, Zou QM.** 2015. A pro-inflammatory role for Th22 cells in *Helicobacter pylori*-associated gastritis. Gut **64:**1368–1378. http://dx.doi.org/10.1136/gutjnl-2014-307020.
50. **Soehnlein O, Dreschsler M, Doring Y, Lievens D, Hartwig H, Kemmerich K, Ortega-Gomez A, Mandl M, Vijayan S, Projahn D, Garlichs CD, Koemem RR, Hristov M, Lutgens E, Zernecke A, Weber C.** 2013. Distinct functions of chemokine receptor axes in the atherogenic mobilization and recruitment of classical monocytes. EMBO Mol Med **5:**471–481. http://dx.doi.org/10.1002/emmm.201201717.
51. **Hang L, Blum AM, Setiawan T, Urban JP, Jr, Stoyanoff KM, Weinstock JV.** 2013. *Heligmosomoides polygyrus bakeri* infection activates colonic $Foxp3^+$ T cells enhancing their capacity to prevent colitis. J Immunol **191:**1927–1934. http://dx.doi.org/10.4049/jimmunol.1201457.
52. **Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ.** 2007. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat Immunol **8:**239–245. http://dx.doi.org/10.1038/ni1443.
53. **Smith P, Walsh CM, Mangan NE, Fallon RE, Sayers JR, McKenzie AN, Fallon PG.** 2004. *Schistosoma mansoni* worms induce anergy of T cells via selective up-regulation of programmed death ligand 1 on macrophages. J Immunol **173:**1240–1248. http://dx.doi.org/10.4049/jimmunol.173.2.1240.
54. **Terrazas LI, Montero D, Terrazas CA, Reyes JL, Rodriguez-Sosa M.** 2005. Role of the programmed death-1 pathway in the suppressive activity of alternatively activated macrophages in experimental cysticercosis. Int J Parasitol **35:**1349–1358. http://dx.doi.org/10.1016/j.ijpara.2005.06.003.
55. **Girgis NM, Gundra UM, Ward LN, Cabrera M, Frevert U, Loke P.** 2014. $Ly6C^{high}$ monocytes become alternatively activated macrophages in schistosome granulomas with help from $CD4^+$ cells. PLoS Pathog **10:** e1004080. http://dx.doi.org/10.1371/journal.ppat.1004080.
56. **Zhang J, Wang B, Zhang W, Wei Y, Bian Z, Zhang CY, Li L, Zen K.** 2013. Protein tyrosine phosphatase 1B deficiency ameliorates murine experimental colitis via the expansion of myeloid-derived suppressor cells. PLoS One **8:**e70828. http://dx.doi.org/10.1371/journal.pone.0070828.
57. **Singh UP, Singh NP, Singh B, Hofseth LJ, Taub DD, Price RL, Nagarkatti M, Nagarkatti PS.** 2012. Role of resveratrol-induced $CD11b^+$ $Gr\text{-}1^+$ myeloid derived suppressor cells (MDSCs) in the reduction of $CXCR3^+$ T cells and amelioration of chronic colitis in $IL\text{-}10^{-/-}$ mice. Brain Behav Immun **26:**72–82. http://dx.doi.org/10.1016/j.bbi.2011.07.236.
58. **Guan Q, Moreno S, Qing G, Weiss CR, Lu L, Bernstein CN, Warrington RJ, Ma Y, Peng Z.** 2013. The role and potential therapeutic application of myeloid-derived suppressor cells in TNBS-induced colitis. J Leukoc Biol **94:**803–811. http://dx.doi.org/10.1189/jlb.0113050.
59. **Zhang R, Ito S, Nishio N, Cheng Z, Suzuki H, Isobe KI.** 2011. Dextran sulphate sodium increases splenic $Gr1^+$ $CD11b^+$ cells which accelerate recovery from colitis following intravenous transplantation. Clin Exp Immunol **164:**417–427. http://dx.doi.org/10.1111/j.1365-2249.2011.04374.x.
60. **Geissmann F, Jung S, Littman DR.** 2003. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity **19:**71–82. http://dx.doi.org/10.1016/S1074-7613(03)00174-2.
61. **Lopes F, Matisz C, Reyes JL, Jijon H, Al-Darmaki A, Kaplan GG, McKay DM.** 2016. Helminth regulation of immunity: a three-pronged approach to treat colitis. Inflamm Bowel Dis **22:**2499–2512. http://dx.doi.org/10.1097/MIB.0000000000000889.
62. **Esser-von Bieren J, Volpe B, Sutherland DB, Burgi J, Verbeek JS, Marsland BJ, Urban JF, Jr, Harris NL.** 2015. Immune antibodies and helminth products drive CXCR2-dependent macrophage-myofibroblast crosstalk to promote intestinal repair. PLoS Pathog **11:**e1004778. http://dx.doi.org/10.1371/journal.ppat.1004778.